

APPLICATION  
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TITLE: ALPHA 1,2 FUCOSYLTRANSFERASE

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## $\alpha$ 1,2-FUCOSYLTRANSFERASE

### FIELD OF THE INVENTION

The present invention relates generally to the field of  $\alpha$ 1,2-fucosyltransferases and,  
5 more specifically, to  $\alpha$ 1,2-fucosyltransferase polypeptides.

### BACKGROUND OF THE INVENTION

*Helicobacter pylori* is an important human pathogen which causes both gastric and duodenal ulcers and has also been associated with gastric cancer and lymphoma. This microorganism has been shown to express cell surface glycoconjugates including  
10 Lewis X, Lewis Y, and sialyl Lewis X. These bacterial oligosaccharides are structurally similar to tumor-associated carbohydrate antigens found in mammals.

The presence of *H. pylori* isolate has been associated with an increased risk for development of gastric cancer (Wirth, H.-P., Yang, M., Karita, M., and Blaser, M. J. (1996) *Infect. Immun.* 64, 4598-4605). This pathogen is highly adapted to colonize  
15 human gastric mucosa and may remain in the stomach with or without causing symptoms for many years. Although *H. pylori* elicits local as well as systemic antibody responses, it escapes elimination by the host immune response due to its sequestered habitation within human gastric mucosa. Another mechanism by which *H. pylori* may protect itself from the action of the host immune response is the  
20 production of surface antigens mimicking those in the host.

In mammalian cells the enzyme fucosyltransferase (namely FucT) catalyzes the last step in the synthesis of two carbohydrate structures, Gal $\beta$  1-4[Fuc $\alpha$ 1-3] GlcNAc (Lewis X, Le<sup>x</sup> for short) or NeuAc $\alpha$ 2-3-Gal $\beta$  1-4[Fuc $\alpha$ 1-3]GlcNAc (sialyl Lewis X, sLe<sup>x</sup> for short). (Lowe *et al.*, 1990, *Cell* 57: 475-484.; Kukowska-Latallo *et al.*, 1990,  
25 *Genes & Development* 4:1288-1303.) Cell surface  $\alpha$ (1,3)- and  $\alpha$ (1,2)-fucosylated oligosaccharides, that is, Lewis X (Le<sup>x</sup>), sialyl Lewis X (sLe<sup>x</sup>) and Lewis Y (Le<sup>y</sup>), are present on both eukaryotic and microbial cell surfaces. In mammals, Le<sup>x</sup> is a

stage-specific embryonic antigen, however,  $\text{Le}^x$ ,  $\text{sLe}^x$  and  $\text{Le}^y$  are also regarded as tumor-associated markers. The biological functions of these bacterial oligosaccharide structures are not fully understood. It has been suggested that such glycoconjugates produced by *H. pylori*, may mimic host cell antigens and could mask the bacterium  
5 from the host immune response. It is also possible that these bacterial Lewis antigens could down regulate the host T-cell response. Therefore, production of such antigens may contribute to colonization and long-term infection of the stomach by *H. pylori*.

Presently, use of carbohydrates as potential therapeutic drugs has become popular in the field of medical chemistry. In addition, qualitative and quantitative carbohydrates  
10 including  $\text{Le}^x$ ,  $\text{Le}^y$  and  $\text{sLe}^x$  are also required as reagents for assaying the enzymes which are involved in the biosynthesis of glycoconjugates in cells.  $\text{Le}^x$ ,  $\text{Le}^y$  and  $\text{sLe}^x$  products which are commercially available are chemically synthesized. However, synthesis of these products gives rise to several limitations such as time-consuming, complicated procedures and low yields. Although several mammalian  
15 fucosyltransferases have been cloned and expressed, enzymatic synthesis of  $\text{Le}^x$ ,  $\text{Le}^y$  and  $\text{sLe}^x$  products for a commercial purpose has not been reported.

The whole genome sequence of *H. pylori* 26695 had been published, which will undoubtedly facilitate the genetic studies of *H. pylori*. *H. pylori* genome sequence revealed the existence of two copies of  $\alpha(1,3)$  *fucT* gene, whereas no putative  $\alpha(1,2)$   
20 *fucT* gene had been annotated.

### SUMMARY OF THE INVENTION

The present invention is based on the discovery of a  $\alpha 1,2$ -fucosyltransferase polypeptide and gene which encodes the polypeptide. The gene was expressed *in vitro* and a mutagenesis study demonstrated that this gene is involved in  $\text{Le}^y$   
25 synthesis. The present invention includes a polynucleotide sequence encoding  $\alpha 1,2$ -fucosyltransferase polypeptide which is useful in the detection and synthesis of

$\alpha$ 1,2-fucosyltransferase polypeptide, and an  $\alpha$ 1,2-fucosyltransferase that is able to synthesize Le<sup>Y</sup>, Le<sup>B</sup> and H type 1 structures.

- Helicobacter pylori* lipopolysaccharide (LPS) express human oncofetal antigens Lewis X and Lewis Y. The synthesis of Lewis Y involves the actions of  $\alpha$ (1,3) and  $\alpha$ (1,2) fucosyltransferases (FucTs). Disclosed herein are the molecular cloning and characterization of genes encoding *H. pylori*  $\alpha$ (1,2) FucT (*Hp fucT2*) from various *H. pylori* strains. Also provided are constructed *Hp fucT2* knock-out mutants that demonstrate the loss of Lewis Y production in these mutants by ELISA and immunoelectron microscopy. The  *$\alpha$ 1,2 fucT2* gene contains a hypermutable sequence (poly C and TAA repeats) which provides a possibility of frequent shifting into and out of coding frame by a polymerase slippage mechanism. Thus,  *$\alpha$ 1,2 fucT2* gene displays two major genotypes: either encoding a single full-length open reading frame (ORF, as in the strain UA802), or truncated ORFs (as in the strain 26695). *In vitro* expression of *Hp fucT2* genes demonstrated that both types of the gene have a potential to produce the full-length protein. The production of the full-length protein by the 26695 *fucT2* gene could be attributed to translational - 1 frameshifting, since a perfect translation frameshift cassette resembling that of *Escherichia coli dnaX* gene is present. The examination of the strain UA1174 revealed that its *fucT2* gene has a frameshifted ORF at the DNA level which cannot be compensated by translation frameshifting, accounting for its Lewis Y -off phenotype. In another strain, UA1218, the *fucT2* gene is turned off apparently due to the loss of its promoter. Based on these data, we proposed a model for the variable expression of Lewis Y by *H. pylori*, in which the regulation at the level of replication, transcription, and translation of the *fucT2* gene may all be involved.
- 25 In another embodiment, the invention provides a method of using the novel  $\alpha$ 1,2-fucosyltransferase to synthesize oligosaccharides such as Le<sup>x</sup>, Le<sup>y</sup>, sLe<sup>x</sup>, Le<sup>A</sup>, Le<sup>B</sup>, H type 1 and H type 2.

In another embodiment the invention provides the novel polypeptide of  $\alpha$ 1,2-fucosyltransferase which is useful in the development of antibodies to  $\alpha$ 1,2-fucosyltransferase.

In another embodiment, a polypeptide of  $\alpha$ 1,2-fucosyltransferase having a frameshift  
5 variant resulting from a "slippery" heptanucleic acid sequence X XXY YYZ, wherein  
X = C or A, Y = T or A and Z = A or G (e.g., A AAA AAG) is provided. In another  
embodiment, the  $\alpha$ 1,2-fucosyltransferase is a polypeptide which has a sequence of  
SEQ ID NO:2. In another embodiment the polynucleotide sequence encoding  
 $\alpha$ 1,2-fucosyltransferase has a variable number of poly-cytosine repeats and TAA  
10 repeats in different *H. pylori* strains.

Further provided is a method for producing  $\alpha$ 1,2-fucosyltransferase. The method  
involves the step of culturing a gene expression system which comprises a host cell  
which has been recombinantly modified with a polynucleotide encoding  
 $\alpha$ 1,2-fucosyltransferase or a portion thereof and harvesting the  $\alpha$ 1,2-  
15 fucosyltransferase. A preferred embodiment of the method is directed to the use of  
the claimed genetic expression system which produces  $\alpha$ 1,2-fucosyltransferase.

Further provided is a method to measure the enzymatic activity and acceptor  
specificity of  $\alpha$ 1,2-fucosyltransferase. The method involves the use of a structurally  
defined oligosaccharide substrate (acceptor) in a radioactive labeled assay system and  
20 identification of the reaction products by capillary electrophoresis. In another  
embodiment, an  $\alpha$ 1,2-fucosyltransferase has a substrate specificity that is distinct  
from the conventional  $\alpha$ 1,2-fucosyltransferase of mammalian origin and uses a  
different pathway to synthesize Lewis antigens.

Also provided are knockout organisms in which expression of  $\alpha$ 1,2-  
25 fucosyltransferase has been prevented or in which the  $\alpha$ 1,2-fucosyltransferase  
expression results in a polypeptide lacking wild type biological activity.

These and many other features and attendant advantages of the present invention will become better understood by reference to the following detailed description of the invention when taken in conjunction with the Examples.

### ABBREVIATIONS

- 5 The abbreviation used are:  $\alpha$ 1,2-FucT,  $\alpha$ 1,2-fucosyltransferase unless specified otherwise; Le<sup>x</sup>, Lewis X; sLe<sup>x</sup>, sialyl-Lewis X; Le<sup>y</sup>, Lewis Y; Le<sup>B</sup>, Lewis B; nt, nucleotide (s); kb, kilobase (s); aa, amino acid (s); PCR, polymerase chain reaction; ORF, open reading frame; RSB, a ribosomal binding site; LPS, lipopolysaccharides; LacNAc-R, Gal $\beta$ 1-4GlcNAc $\beta$ -O-(CH<sub>2</sub>)<sub>8</sub>COOMe; Gal $\beta$ 1-3GlcNAc-R,
- 10 Gal $\beta$ 1-3GlcNAc $\beta$ -O-(CH<sub>2</sub>)<sub>8</sub>COOMe; LacNAc-TMR, Gal $\beta$ 1-4GlcNAc $\beta$ -O-(CH<sub>2</sub>)<sub>8</sub>CO-NHCH<sub>2</sub>CH<sub>2</sub>NH-TMR.

### BRIEF DESCRIPTION OF THE DRAWINGS

- Fig. 1 shows the nucleotide sequence analysis of *Hp fucT2*. (A) Gene organization of *Hp fucT2* region in the genome of *H. pylori* 26695 and UA802. GW44 and GW32
- 15 indicate the two primers used for cloning *Hp fucT2* genes. (B) Nucleotide sequences of the center region of *Hp fucT2* showing the features (simple repeats) responsible for frameshift between prototype (UA802) and variant type (26695) genes. The divergent nucleotides between the two sequences are marked by "x". Due to the different repeat number of poly C residues, the initiating reading frame of 26695 *fucT2* (HP0094)
- 20 encounters a TGA stop codon (marked with asterisks) shortly after the poly-C region. About 110 bp further downstream, there appears a potential start codon ATG (marked with dots) in the -1 frame (HP0093), which is the same as the reading frame of 802 *fucT2*. The three putative X XXY YYZ motifs (X, Y, and Z represent specific nucleotides in a particular reading from) are given in bold face and underlined.
- 25 Additional elements for programmed translation frameshift in 26695 *fucT2* resembling those in *E. coli dnaX* gene are also underlined. (C) The putative 26695 *fucT2* translation frameshift cassette. Shown is the mRNA structure deduced from the DNA sequence in line 2 of (B). The AAAAAAG heptamer (bold) is a highly slippery

sequence identified in other DNA sequences. UGA (sidelined in the stem structure) is the stop codon in the initiating frame (0 frame). SD indicates an internal Shine-Dalgarno-like sequence. According to the *E. coli dnaX* frameshift model, AAAAAAG sequence is the frameshift site, and both upstream SD sequence and downstream stem-loop structure enhance frameshifting. (D) Shows the amino acid sequence and nucleic acid sequence for  $\alpha$ 1,2 fucosyltransferase.

Fig. 2 shows an analysis of the deduced Amino acid (aa) sequence of *Hp fucT2*. (A) Schematic representation of the domain structures of mammalian and bacterial  $\alpha$ (1,2) fucosyltransferases. Cyt, cytoplasmic. TM, transmembrane. Hatched boxes represent three highly conserved aa sequence motifs. (B) Alignment of the three motifs of aa sequences which are highly conserved in all prokaryotic and eukaryotic  $\alpha$ (1,2) fucosyltransferases. The length (in aa) of each protein is given in parentheses after the name of organisms, and the positions of each motif within the protein are labeled in parentheses after each amino acid sequence. Ye, *Y. enterocolitica*. Ll, *Lactococcus lactis*. Accession numbers of these sequences are: M35531 (man FUT1), U17894 (man FUT2), AF076779 (Hp FucT2, from the prototype *fucT2* of UA802), U46859 (Ye WbsH), and U93364 (Ll EpsH).

Fig. 3 shows the cloning and *in vitro* expression of *Hp fucT2* genes. (A) Plasmid constructs containing intact or partial *Hp fucT2* gene. Heavy arrows represent the predicted ORFs, and the thin lines indicate the flanking regions that had been cloned together with the coding region into the vector. The small arrows point to the direction of the transcription from the T7 promoter. Restriction endonuclease sites *Hind*III (H) and *Eco*RI (E) were used for constructing CAT insertion mutants. (B) Autoradiograph of a 0.1% SDS - 12% PAGE analyzing the protein synthesis products from various plasmid constructs by *E. coli* T7 S30 extract. Lane 1, no DNA template. Some protein bands are from transcription-translation of endogenous DNA or RNA in the cell extract. Lane 2, pGEM-T vector. Lane 3, 4, and 5, plasmid constructs pGEMB3, pGEMH2, and pGEMI6, respectively. The full length protein (33 KD)

marked by the large arrow was overexpressed from intact *fucT2* genes but not from 5'-truncated gene. A half-length protein (17 KD, marked by the small arrow) was also produced from 26695 *fucT2*, but not from 802 *fucT2*. Lane 6, 7, and 8, pGHC26, pGEC26, and pGHC8, plasmid mutants with CAT insertion at *Hind*III site of 26695 *fucT2*, at *Eco*RI site of 26695 *fucT2*, and at *Hind*III site of UA802 *fucT2*, respectively. All three plasmid mutants gave rise to strong expression of 24 KD CAT protein. The molecular mass markers (Life Technologies, Inc) are indicated on the right.

Fig. 4 shows a transmission electron micrographs of *H. pylori* UA802 and its isogenic mutant carrying CAT insertion within the *fucT2* gene at *Hind*III ( $\Delta$ H). Cells were incubated with anti-Le<sup>Y</sup> MAb and goat anti-mouse IgM-10nm colloidal gold particles. Gold particles were present on the wild type cell (both on the cell wall and flagella sheath, marked by arrowheads) but absent on the mutant cell.

Fig. 5 shows two possible pathways for the synthesis of Lewis Y in *H. pylori*.

Fig. 6 shows an immunoblots of *H. pylori* LPS for detection of Lewis structures. Proteinase K treated whole cells extracts of *H. pylori* 26695 and UA802 wild type strains (WT) and their isogenic mutants ( $\Delta$ H and  $\Delta$ E) were resolved on SDS-PAGE and electroblotted onto a nitrocellulose membrane, and the LPS were immunostained using anti-Le<sup>Y</sup> (A) or anti-Le<sup>X</sup> antibody (B).

Fig. 7 shows identification of the reaction products of Hp  $\alpha$ 1,2-fucosyltransferase by capillary electrophoresis. The enzyme used here was the overexpressed UA802  $\alpha$ 1,2-fucosyltransferase polypeptide. The reactions were carried out as described in Example 3 below. (A) The reaction of type 2 substrates LacNAc (line a) and Le<sup>X</sup> (line b). (B) The reactions on Type 1 substrates (line d) and Le<sup>B</sup> (line e). Line c and f represent the standard TMR-labeled oligosaccharides: (1) linking arm, (2) GlcNAc, (3) LacNAc, (4) H type 2, (5) Le<sup>X</sup>, (6) Le<sup>Y</sup>, (7) Type 1, (8) H type 1, (9) Le<sup>A</sup>, and (10) Le<sup>B</sup>. All electropherograms are Y-offset for clarity.



Fig. 8 shows identified pathways for the synthesis of Lewis antigens in *H. pylori*.

Lewis structures known to be expressed on the *H. pylori* cell surface are boxed. Solid arrows represent the fucosyltransferase activities that have been demonstrated in this study, and the thickness of the arrows indicates the relative level of the enzyme

- 5 activity. (A) *H. pylori* strains predominantly express Le<sup>x</sup> and Le<sup>y</sup>, and do not appear to express H type 2. It seems reasonable that *H. pylori* utilizes Le<sup>x</sup> to synthesize Le<sup>y</sup>. For operation of this pathway *H. pylori* normally maintains a higher level of  $\alpha$ 1,3-fucosyltransferase than of  $\alpha$ 1,2-fucosyltransferase. (B) *H. pylori*  $\alpha$ 1,2-fucosyltransferase has the ability to transfer fucose to Type 1 as well as to Le<sup>a</sup>. The
- 10 synthesis of Le<sup>b</sup> requires the concerted action of  $\alpha$ 1,2-fucosyltransferase with an  $\alpha$ 1,4-fucosyltransferase.

### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to a purified  $\alpha$ 1,2-fucosyltransferase polypeptide, polynucleotide which encode the  $\alpha$ 1,2-fucosyltransferase, and the use of the

- 15  $\alpha$ 1,2-fucosyltransferase gene and  $\alpha$ 1,2-fucosyltransferase polypeptide in the production of biologics and in the screening of biological tissues and fluids. The invention also relates to antibodies against  $\alpha$ 1,2-fucosyltransferase polypeptides and their use in diagnosing disorders and in monitoring disease.

#### **The $\alpha$ 1,2-fucosyltransferase Polypeptide**

The amino acid sequence encoded by the  $\alpha$ 1,2-fucosyltransferase gene is shown in Fig 1D (SEQ ID NO:2). Because the  $\alpha$ 1,2-fucosyltransferase are prokaryotically derived post-translational modifications are not made to the enzyme, unlike the eukaryotically expressed  $\alpha$ 1,2-fucosyltransferase.

- 25 Additionally, the  $\alpha$ 1,2-fucosyltransferase polypeptide may be altered by addition, substitution or deletions of peptide sequences in order to modify its activity. For example, polypeptide sequences may be fused to the  $\alpha$ 1,2-fucosyltransferase polypeptide in order to effectuate additional enzymatic activity. Alternatively, amino

acids may be deleted or substituted to remove or modify the activity of the protein. The protein may be modified to lack  $\alpha$ 1,2-fucosyltransferase enzymatic activity, but retain its three-dimensional structure. Such modification would be useful in the development of antibodies against  $\alpha$ 1,2-fucosyltransferase polypeptide as described  
5 more fully below.

In yet another embodiment, the invention includes aspects of the enzymatic activity of  $\alpha$ 1,2-fucosyltransferase, wherein the  $\alpha$ 1,2-fucosyltransferase polypeptide lacks  $\alpha$ 1,4-fucosyltransferase or  $\alpha$ 1,3-fucosyltransferase activity or lacks both  $\alpha$ 1,3-fucosyltransferase and  $\alpha$ 1,4-fucosyltransferase activity.

10 The  $\alpha$ 1,2-fucosyltransferase gene product may include those polypeptides encoded by the  $\alpha$ 1,2-fucosyltransferase gene sequences described in the section below.

Specifically,  $\alpha$ 1,2-fucosyltransferase gene products, sometimes referred to herein as " $\alpha$ 1,2-fucosyltransferase polypeptide", may include  $\alpha$ 1,2-fucosyltransferase gene product encoded by an  $\alpha$ 1,2-fucosyltransferase gene sequence shown in Fig. 1 and  
15 SEQ ID NO:1, as well as different versions of the gene sequences deposited in GenBank under the accession numbers AF093828 - AF093833. Thus, the term " $\alpha$ 1,2-fucosyltransferase polypeptide" includes full length expression as well as polypeptides, such as smaller peptides, which retain a biological activity of the full length product, such as  $\alpha$ 1,2-fucosyltransferase activity.

20 In addition,  $\alpha$ 1,2-fucosyltransferase gene products may include proteins or polypeptides that represent functionally equivalent gene products. Such an equivalent  $\alpha$ 1,2-fucosyltransferase gene product may contain deletions, additions or substitutions of amino acid residues within the amino acid sequence encoded by the  $\alpha$ 1,2-fucosyltransferase gene sequences described above, but which results in a silent  
25 change, thus producing a functionally equivalent  $\alpha$ 1,2-fucosyltransferase gene product. Amino acid substitutions may be made on the basis of similarity in polarity,

charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; planar neutral  
5 amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Functionally equivalent", as utilized herein, refers to a polypeptide capable of exhibiting a substantially similar *in vivo* activity as the endogenous  
10  $\alpha$ 1,2-fucosyltransferase gene products encoded by the  $\alpha$ 1,2-fucosyltransferase gene sequences described above, as judged by any of a number of criteria, including but not limited to antigenicity, *i.e.*, the ability to bind to an anti- $\alpha$ 1,2-fucosyltransferase antibody, immunogenicity, *i.e.*, the ability to generate an antibody which is capable of binding a  $\alpha$ 1,2-fucosyltransferase protein or polypeptide, as well as enzymatic  
15 activity. For example, the frameshift mutant resulting from expression of the sequence XXXYYYZ results in a product which may retain antigenic properties similar to those of wild type  $\alpha$ 1,2-fucosyltransferase.

A substantially purified  $\alpha$ 1,2-fucosyltransferase protein, polypeptide, and derivative (including a fragment) is substantially free of other proteins, lipids, carbohydrates,  
20 nucleic acids, and other biological materials with which it is naturally associated. For example, a substantially purified functional fragment of  $\alpha$ 1,2-fucosyltransferase polypeptide can be at least 60%, by dry weight, the molecule of interest. One skilled in the art can purify a functional fragment of  $\alpha$ 1,2-fucosyltransferase protein using standard protein purification methods and the purity of the polypeptides can be  
25 determined using standard methods including, *e.g.*, polyacrylamide gel electrophoresis (*e.g.*, SDS-PAGE), column chromatography (*e.g.*, high performance liquid chromatography), and amino-terminal amino acid sequence analysis.

- Included within the scope of the invention are  $\alpha$ 1,2-fucosyltransferase proteins, polypeptides, and derivatives (including fragments) which are differentially modified during or after translation. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; *etc.* Additionally, the composition of the invention may be conjugated to other molecules to increase their water-solubility (e.g., polyethylene glycol), half-life, or ability to bind targeted tissue.
- 5
- 10 Furthermore, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the  $\alpha$ 1,2-fucosyltransferase polypeptide sequence. Non-classical amino acids include, but are not limited to, the D-isomer of the common amino acids,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu, epsilon-Ahx, 6-amino hexanoic acid, Aib, 2-amino
- 15 isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids, such as  $\beta$ -methyl amino acids,  $\alpha$ -methyl amino acids,  $N\alpha$ -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D
- 20 (dextrorotary) or L (levorotary).

- While random mutations can be made to  $\alpha$ 1,2-fucosyltransferase DNA (using random mutagenesis techniques known to those skilled in the art) and the resulting mutant  $\alpha$ 1,2-fucosyltransferase polypeptides tested for activity, site-directed mutation of the  $\alpha$ 1,2-fucosyltransferase coding sequence can be engineered (using site-directed
- 25 mutagenesis techniques well known to those skilled in the art) to create mutant  $\alpha$ 1,2-fucosyltransferase polypeptides with increased functional characteristics.

- Polypeptides corresponding to one or more domains of the  $\alpha$ 1,2-fucosyltransferase protein, truncated or deleted  $\alpha$ 1,2-fucosyltransferase proteins, as well as fusion proteins in which the full length  $\alpha$ 1,2-fucosyltransferase proteins, polypeptides, or derivatives (including fragments), or truncated  $\alpha$ 1,2-fucosyltransferase, is fused to an unrelated protein, are also within the scope of the invention and can be designed on the basis of the  $\alpha$ 1,2-fucosyltransferase nucleotide and  $\alpha$ 1,2-fucosyltransferase amino acid sequences disclosed in this section and the section above. The fusion protein may also be engineered to contain a cleavage site located between a  $\alpha$ 1,2-fucosyltransferase sequence and the non- $\alpha$ 1,2-fucosyltransferase protein sequence, so that the  $\alpha$ 1,2-fucosyltransferase polypeptide may be cleaved away from the non- $\alpha$ 1,2-fucosyltransferase moiety. Such fusion proteins or polypeptides include but are not limited to IgFc fusion which may stabilize the  $\alpha$ 1,2-fucosyltransferase protein *in vivo*; or fusion to an enzyme, fluorescent protein, or luminescent protein which provide a marker function.
- 15 The  $\alpha$ 1,2-fucosyltransferase polypeptide may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the  $\alpha$ 1,2-fucosyltransferase polypeptides of the invention by expressing a nucleic acid containing  $\alpha$ 1,2-fucosyltransferase gene sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing  $\alpha$ 1,2-fucosyltransferase coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook *et al.*, 1989, *Molecular Cloning, a Laboratory Manual*, Cold Springs Harbor Press, N.Y., and Ausubel F.M. *et al.*, eds., 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc., and John Willey & Sons, Inc., New York.
- 25 Alternatively, RNA capable of encoding  $\alpha$ 1,2-fucosyltransferase polypeptide may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press,

oligosaccharides are produced the  $\alpha$ 1,2-fucosyltransferase polypeptide may be inactivated by heating, centrifugal separation, or the like. The resulting fucosylated oligosaccharides may be further purified by techniques known to those skilled in the art.

- 5 Cell containing systems for the synthesis of fucosylated oligosaccharides may include recombinantly modified host cells according to the methods described below or may be naturally occurring cells which express  $\alpha$ 1,2-fucosyltransferase polypeptide or an enzymatically active portion thereof, so long as the cell is capable of catalyzing the synthesis of fucosylated oligosaccharides from substrates.
- 10 In the case of cell containing systems the host cell is contacted with the substrate, under conditions and for sufficient time to produce the oligosaccharide. The time and conditions will vary depending upon the host cell type and culture conditions and can be easily determined by those of skill in the art.

- The invention provides a gene expression system for producing  $\alpha$ 1,2-
- 15 fucosyltransferase polypeptides. The gene expression system comprises a host cell which has been modified with a polynucleotide encoding  $\alpha$ 1,2-fucosyltransferase polypeptide or a portion thereof, as described above.

A preferred gene expression system of the invention involves host cell modified with a polynucleotide encoding  $\alpha$ 1,2-fucosyltransferase polypeptide or a portion thereof.

- 20 The method involves culturing a gene expression system created according to the methods described above under conditions sufficient to produce the  $\alpha$ 1,2-fucosyltransferase polypeptide. The gene expression system comprises a host cell which has been recombinantly modified with a polynucleotide encoding a  $\alpha$ 1,2-fucosyltransferase polypeptide or a portion thereof.

The method is also directed to harvesting the  $\alpha$ 1,2-fucosyltransferase polypeptide. A further step of the method involves substantially purifying the harvested  $\alpha$ 1,2-fucosyltransferase. The purified  $\alpha$ 1,2-fucosyltransferase polypeptide may be used in the synthesis of fucosylated oligosaccharides or the preparation of antibodies as  
5 described above.

Specifically disclosed herein is a gene expression system recombinantly modified with a DNA sequence containing the  $\alpha$ 1,2-fucosyltransferase gene. The sequence contains an open reading frame (ORF) of approximately 900 base pairs which are transcribed into  $\alpha$ 1,2-fucosyltransferase product having a calculated molecular weight  
10 of 35,193 daltons.

As used herein, the term "recombinantly modified" means introducing a polynucleotide encoding  $\alpha$ 1,2-fucosyltransferase polypeptide into a living cell or gene expression system. Usually, the polynucleotide is present in a plasmid or other vector, although modification can also occur by uptake of free  $\alpha$ 1,2-fucosyltransferase  
15 polynucleotide or numerous other techniques known in the art.

As used herein, the term "gene expression system" means a living eukaryotic or prokaryotic cell into which a gene, whose product is to be expressed, has been introduced, as described above.

As used herein, the term "harvesting" means collecting or separating from the gene  
20 expression system the product produced by the inserted polynucleotide.

Polynucleotide sequences encoding  $\alpha$ 1,2-fucosyltransferase polypeptides can be expressed by polynucleotide transfer into a suitable host cell.

"Host cells" are cells in which a vector can be propagated and its DNA expressed. A gene expression system is comprised of a host cell in which a vector was propagated

and the vector's DNA expressed. The term "host cell" also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Host cells which are  
5 useful in the claimed gene expression system and the claimed method of producing  $\alpha$ 1,2-fucosyltransferase polypeptide include bacterial cells, yeast cells fungal cells, plant cells and animal cells.

Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art. In the present invention, the  $\alpha$ 1,2-fucosyltransferase  
10 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the  $\alpha$ 1,2-fucosyltransferase genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence  
15 of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

20 The method of the invention produces  $\alpha$ 1,2-fucosyltransferase polypeptide which are substantially pure. As used herein, the term "substantially pure" refers to a protein which is free of other proteins, lipids, carbohydrates or other materials with which it is normally associated. One skilled in the art can purify  $\alpha$ 1,2-fucosyltransferase polypeptide using standard techniques for protein purification including preparative  
25 chromatography and immunological separations involving monoclonal or polyclonal antibodies. For example, the substantially pure  $\alpha$ 1,2-fucosyltransferase protein will yield a single major band of approximately 35 kD on a non-reducing polyacrylamide gel. The purity of the  $\alpha$ 1,2-fucosyltransferase polypeptide can also be determined by



Oxford, which is incorporated by reference herein in its entirety. The use of such synthetic peptide fragments of  $\alpha$ 1,2-fucosyltransferase for generating polyclonal antibodies is described below.

#### The $\alpha$ 1,2-fucosyltransferase Gene

- 5 The  $\alpha$ 1,2-fucosyltransferase gene (Fig. 1) is expressed in *H. pylori*. Nucleic acid sequences of the identified  $\alpha$ 1,2-fucosyltransferase genes are described herein. As used herein, " $\alpha$ 1,2-fucosyltransferase gene" refers to (a) a gene containing the DNA sequence shown in Fig. 1; (b) any DNA sequence that encodes the amino acid sequence shown in Fig. 1D, SEQ ID NO: 2; (c) any DNA sequence that hybridizes to
- 10 the complement of the coding sequences shown in Fig. 1, SEQ ID NO: 1, under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, eds., 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc., and John Willey & Sons, Inc.,
- 15 New York, at p. 2.10.3) and encodes a gene product functionally equivalent to a gene product encoded by sequences shown in Fig. 1; and/or (d) any DNA sequence that hybridizes to the complement of the coding sequences disclosed herein (as shown in Fig. 1), under less stringent conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2% SSC/0.1% SDS at 42°C (Ausubel *et al.*, 1989, *supra*), and encodes a
- 20 gene product functionally equivalent to a gene product encoded by sequences shown in Fig. 1.

- The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the DNA sequences (a) through (c), in the preceding paragraph. Such hybridization conditions may be highly
- 25 stringent or less highly stringent, as described above. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), highly stringent conditions may refer, *e.g.*, to washing in 6xSSC/0.05% sodium pyrophosphate at 37 °C (for 14-base oligos), 48 °C (for 17-base oligos), 55 °C (for 20-base oligos), and 60 °C (for 23-base

oligos). These nucleic acid molecules may act at  $\alpha$ 1,2-fucosyltransferase gene regulation and/or as antisense primers in amplification reactions of  $\alpha$ 1,2-fucosyltransferase gene nucleic acid sequences. Further, such sequences may be used as part of ribozyme and/or triple helix sequences, also useful for

5  $\alpha$ 1,2-fucosyltransferase gene regulation. Still further, such molecules may be used as components of diagnostic methods whereby the presence of a pathogen or metastatic tumor cell may be detected.

The invention also encompasses (a) DNA vectors that contain any of the foregoing coding sequences and/or their complements (*e.g.*, antisense); (b) DNA expression

10 vectors that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences; and (c) genetically engineered host cells that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell. As used herein, regulatory elements include, but

15 are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression.

The invention includes fragments of any of the DNA sequences disclosed herein. Fragments of the  $\alpha$ 1,2-fucosyltransferase gene corresponding to coding regions of particular domains, or in which one or more of the coding regions of the domains is

20 deleted, are useful. Such  $\alpha$ 1,2-fucosyltransferase gene fragments may encode truncated gene products that retain a biological activity of the full-length  $\alpha$ 1,2-fucosyltransferase polypeptide, such as  $\alpha$ 1,2-fucosyltransferase activity or immunogenicity. The invention also includes mutant  $\alpha$ 1,2-fucosyltransferase genes encoding substitutions of amino acids as described below.

25 In addition to the gene sequences described above, homologs of such sequences, as may, for example, be present in other species, including humans, may be identified and may be readily isolated, without undue experimentation, by molecular biological

techniques well known in the art. Further, there may exist genes at other genetic loci within the genome that encode proteins which have extensive homology to one or more domains of such gene products. These genes may also be identified via similar techniques.

- 5 The  $\alpha$ 1,2-fucosyltransferase gene and its homologs can be obtained from other organisms thought to contain  $\alpha$ 1,2-fucosyltransferase activity. For obtaining cDNA, tissues and cells in which  $\alpha$ 1,2-fucosyltransferase is expressed are optimal. Tissues which can provide a source of genetic material for  $\alpha$ 1,2-fucosyltransferase and its homologs, therefore, include intestinal mucosal cells and tumorigenic cells. For  
10 example, the isolated  $\alpha$ 1,2-fucosyltransferase gene sequences may be labeled and used to screen a cDNA library constructed from mRNA obtained from the organism of interest. The hybridization conditions used should be of a lower stringency when the cDNA library is derived from an organism different from the type of organism from which the labeled sequence was derived. Alternatively, the labeled fragment  
15 may be used to screen a genomic library derived from the organism of interest, again, using appropriately stringent condition. Low stringency conditions are well known in the art, and will vary predictably depending on the specific organism from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook *et al.*, 1989, *Molecular Cloning, a Laboratory*  
20 *Manual*, Cold Springs Harbor Press, N.Y.; and Ausubel *et al.*, 1989, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y.

- Further, a previously unknown  $\alpha$ 1,2-fucosyltransferase gene type sequence may be isolated by performing PCR using two degenerate oligonucleotide primer pools  
25 designed on the basis of amino acid sequence within the gene of interest. The template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from human or non-human cell lines or tissue known or suspected to express a  $\alpha$ 1,2-fucosyltransferase gene.

The PCR product may be subcloned and sequenced to ensure that the amplified sequences represent the sequences of a  $\alpha$ 1,2-fucosyltransferase gene-like nucleic acids sequences. The PCR fragment may then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled  
5 and used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to screen a genomic library.

PCR technology may also be utilized to isolate DNA sequences, including full length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription  
10 reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanidines using a standard terminal transferase reaction, the hybrid may be digested with RNase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA  
15 sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies which may be used, see *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning, a Laboratory Manual*, Cold Springs Harbor Press, N.Y.

In cases where the  $\alpha$ 1,2-fucosyltransferase gene identified is the normal, or wild type, gene, this gene may be used to isolate mutant alleles of the gene. Mutant alleles may  
20 be isolated from individuals either known or proposed to have a genotype which contributes to intestinal mucosal disease and/or tumorigenicity. Mutant alleles and mutant allele products may then be utilized in the therapeutic and diagnostic systems described below.

A cDNA of the mutant gene may be isolated, for example by PCR. In this case, the  
25 first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying the mutant allele, and by extending the new strand with reverse

transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically the 5' end of the normal gene. Using these primers, the product is then amplified via PCR, cloned into a suitable vector, and subjected to DNA sequences analysis through methods known in the art. By

- 5 comparing the DNA sequence of the mutant gene to that of the normal gene, the mutation(s) responsible for the loss or alteration of function of the mutant gene product can be ascertained.

- A variety of host-expression vector systems may be utilized to express the  $\alpha$ 1,2-fucosyltransferase gene coding sequences of the invention. Such host-expression
- 10 systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the  $\alpha$ 1,2-fucosyltransferase gene product of the invention *in situ*. These hosts include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed
- 15 with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing  $\alpha$ 1,2-fucosyltransferase gene product coding sequences; yeast (*e.g.* *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the  $\alpha$ 1,2-fucosyltransferase gene product coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus)
- 20 containing the  $\alpha$ 1,2-fucosyltransferase gene product coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing  $\alpha$ 1,2-fucosyltransferase gene product coding sequences; or mammalian cell systems (*e.g.*, COS, SHO, BHK, 293, 3T3)
- 25 harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the  $\alpha$ 1,2-fucosyltransferase gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of  $\alpha$ 1,2-fucosyltransferase polypeptide or for raising antibodies to  $\alpha$ 1,2-fucosyltransferase polypeptide, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO J.* 2:1791), in which the  $\alpha$ 1,2-fucosyltransferase gene product coding sequence may be ligated individually into the vector in frame with the *lac z* coding region that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109); and the like. pGEX vectors may also be used to express foreign polypeptide as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The  $\alpha$ 1,2-fucosyltransferase gene coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under the control of an AcNPV promoter. Successful insertion of  $\alpha$ 1,2-fucosyltransferase gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus. These recombinant viruses are then used to infect *S. frugiperda* cells in which the inserted gene is expressed.

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the  $\alpha$ 1,2-fucosyltransferase gene coding sequence of interest may be ligated to an adenovirus

- transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of
- 5 expressing  $\alpha$ 1,2-fucosyltransferase gene product in infected hosts (See Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted  $\alpha$ 1,2-fucosyltransferase gene product coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire  $\alpha$ 1,2-fucosyltransferase gene, including
- 10 its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translation control signals may be needed. However, in cases where only a portion of the  $\alpha$ 1,2-fucosyltransferase gene coding sequences is inserted, exogenous translational control signals, including, the ATG initiation codon must be provided.
- 15 Transfection via retroviral vectors, naked DNA methods and mechanical methods including micro injection and electroporation may be used to provide either stably transfected host cells (*i.e.*, host cells that do not lose the exogenous DNA over time) or transient transfected host cells (*i.e.*, host cells that lose the exogenous DNA during cell replication and growth).
- 20 An alternative fusion protein system allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, *et al.*, 1991; *Proc. Natl. Acad. Sci. USA* 88:8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues.
- 25 Extracts from cell infected with recombinant vaccinia virus are loaded onto  $\text{Ni}^{2+}$  nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The  $\alpha$ 1,2-fucosyltransferase gene products can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates may be used to generate  $\alpha$ 1,2-fucosyltransferase transgenic animals.

#### 5 Expression Systems for $\alpha$ 1,2-fucosyltransferase

The novel bacterial  $\alpha$ 1,2-fucosyltransferase encoded by the disclosed gene, and enzymatically active fragment thereof, can be used in the production of fucosylated oligosaccharides such as Lewis Y (Le<sup>Y</sup>) and Lewis B (Le<sup>B</sup>). These bacterial oligosaccharides are structurally similar to certain tumor-associated carbohydrate  
10 antigens found in mammals. These product glycoconjugates also have research and diagnostic utility in the development of assays to detect mammalian tumors.

The fucosylated oligosaccharides may be produced by any number of methods utilizing the methods and compositions described herein. Standard enzymology techniques well known in the art may be utilized to develop systems to provide  
15 fucosylated oligosaccharides (see for example the *Methods in Enzymology*, volume series published by Academic Press; and Tim Bugg, "An Introduction to Enzyme and Coenzyme Chemistry", 1997, Blackwell Sciences, Inc.).

"Substrate", as used herein, means any material or combinations of different materials, that may be acted upon by the polypeptide of the invention to give rise to  
20 fucosylated oligosaccharides, for example, and not by way of limitation, substrates may include LacNAc-R and GDP-fucose.

Cells containing and cell-free systems may be used to produce the fucosylated oligosaccharides of the present invention. Cells containing and cell-free systems will be better understood in the description and examples that follow. Such systems are  
25 useful in the development of fucosylated oligosaccharides.



The present invention provides a method for synthesizing fucosylated oligosaccharides by reacting substrates in the presence of  $\alpha$ 1,2-fucosyltransferase, capable of catalyzing the formation of the fucosylated oligosaccharides from the substrates.

- 5 The  $\alpha$ 1,2-fucosyltransferase may be used regardless of its origin so long as it is capable of producing the fucosylated oligosaccharides from the substrates. The source of the  $\alpha$ 1,2-fucosyltransferase may be derived according to the methods and compositions as described herein, for example, through protein purification from host cells transfected with an expression system as described more fully below.
- 10 The substrates are allowed to react with the  $\alpha$ 1,2-fucosyltransferase polypeptide under suitable conditions to allow formation of the enzymatic product. Suitable conditions can be easily determined by one skilled in the art. For example, suitable conditions will include contacting the substrate and polypeptide for a sufficient time and under sufficient conditions to allow formation of the enzymatic product, *e.g.* Le<sup>y</sup>, Le<sup>B</sup>.
- 15 These conditions will vary depending upon the amounts and purity of the substrate and enzyme, whether the system is a cell-free or cellular based system. These variables will be easily adjusted by those skilled in the art. For example, the period of exposure of the enzyme to the substrate will be longer at lower temperatures, *e.g.*, 4 °C rather than at higher temperatures. In the methods for synthesizing the fucosylated
- 20 oligosaccharides there are no restriction in terms of the timing of the addition of the substrates. The ratios of the various substrates should be in equal proportions, *i.e.* 1:1. The ratios of the enzyme to the substrates may be varied depending upon the rate and quantity of fucosylated oligosaccharides desired.

The method of producing the fucosylated oligosaccharides may be carried out at  
25 temperatures of 4 °C to 60 °C. Additionally, a number of buffers may be used, for example, and not by way of limitation, a buffer having a pH between 6.5 and 8.0, and in the presence of 15-30 mM Mn<sup>2+</sup>. After a desired amount of fucosylated

amino-terminal amino acid sequence analysis.  $\alpha$ 1,2-fucosyltransferase polypeptide include functional fragments of the polypeptide, so long as biological activity remains, such as  $\alpha$ 1,2-fucosyltransferase enzymatic activity. Accordingly, the invention includes a gene expression system and a method of producing  $\alpha$ 1,2-  
5 fucosyltransferase polypeptide which produce smaller peptides containing the enzymatic activity of  $\alpha$ 1,2-fucosyltransferase.

Production of  $\alpha$ 1,2-fucosyltransferase. Production of  $\alpha$ 1,2-fucosyltransferase from the gene expression system of the invention is achieved by culturing a gene expression system comprising a host cell recombinantly modified with a  
10 polynucleotide encoding  $\alpha$ 1,2-fucosyltransferase polypeptide or an enzymatically active portion thereof and harvesting the  $\alpha$ 1,2-fucosyltransferase polypeptide. The method further comprises substantially purifying the harvested  $\alpha$ 1,2-fucosyltransferase polypeptide using protein purification protocols well known in the art (*Current Protocols in Molecular Biology*, Chapter 10, eds. Ausubel, F.M. *et al.*,  
15 1994).

The method for producing  $\alpha$ 1,2-fucosyltransferase polypeptide involves culturing the gene expression system of the invention under conditions of continuous culture, such as, but not restricted to, "fed-batch cultures" or continuous perfusion cultures. Other continuous culture systems which find use in the present invention is set forth in  
20 Wang, G. *et al. Cytotechnology* 9:41-49, 1992; Kadouri, A. *et al. Advances in Animal Cell Biology and Technology for Bioprocesses*, pp. 327-330, Courier International, Ltd., 1989; Spier, R.E. *et al. Biotechnol. Bioeng.* 18:649-57, 1976.

#### Antibodies to $\alpha$ 1,2-fucosyltransferase Proteins

Antibodies that define the  $\alpha$ 1,2-fucosyltransferase gene product are within the scope  
25 of this invention, and include antibodies capable of specifically recognizing one or more  $\alpha$ 1,2-fucosyltransferase gene product epitopes. Such antibodies may include, but are not limited to, polyclonal antibodies, monoclonal antibodies, humanized or

chimeric antibodies, single chain antibodies, Fab fragments, F(ab')<sub>2</sub> fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Such antibodies may be used, for example, in the detection of  $\alpha$ 1,2-fucosyltransferase gene product in a biological sample, including, but not limited to, blood, plasma, and serum. Alternatively, the antibodies may be used as a method for the inhibition of abnormal  $\alpha$ 1,2-fucosyltransferase gene product activity. Thus, such antibodies may be utilized as part of treatment for intestinal mucosal disease, and may be used as part of diagnostic techniques whereby patients may be tested for abnormal levels of  $\alpha$ 1,2-fucosyltransferase gene products, or for the presence of abnormal forms of such proteins.

For the production of antibodies against a  $\alpha$ 1,2-fucosyltransferase gene product, various host animals may be immunized by injection with a  $\alpha$ 1,2-fucosyltransferase gene product, or a portion thereof. Such host animals may include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG, interferon and other cytokines effecting immunological response.

Polyclonal antibodies are a heterogenous population of antibody molecules derived from the sera of animals immunized with an antigen, such as a  $\alpha$ 1,2-fucosyltransferase gene product, or an antigenic functional derivative thereof. In general, for the production of polyclonal antibodies, host animals such as those described above, may be immunized by injection with  $\alpha$ 1,2-fucosyltransferase gene product supplemented with adjuvants as also described above.

Monoclonal antibodies (mAbs), which are homogenous population of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique of Kohler and  
5 Milstein, (1975, Nature 256:495-497; and U.S. Patent No. 4,376,110), human B-cell hybridoma technique (Kosbor *et al.*, 1983, Immunology Today 4:72; Cole *et al.*, 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole *et al.*, 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM,  
10 IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.*, 1984,  
15 Nature, 312:604-608; Takeda *et al.*, 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine  
20 mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242:423-426; Huston *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward *et al.*, 1989, Nature 334:544-546) can be adapted to produce single chain antibodies against  $\alpha$ 1,2-fucosyltransferase gene  
25 products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')<sub>2</sub> fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

#### **Methods of Detecting $\alpha$ 1,2-fucosyltransferase in Biological Samples**

The antibodies described above can be used in the detection of  $\alpha$ 1,2-fucosyltransferase polypeptides in biological samples.  $\alpha$ 1,2-fucosyltransferase polypeptide from blood or other tissue or cell type may be easily isolated using techniques which are well known to those of skill in the art. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety.

Preferred diagnostic method for the detection of wild type or mutant  $\alpha$ 1,2-fucosyltransferase polypeptides may involve, for example, immunoassays wherein  $\alpha$ 1,2-fucosyltransferase polypeptides are detected by their interaction with an anti- $\alpha$ 1,2-fucosyltransferase polypeptide specific antibody.

For example, antibodies, or fragments of antibodies, such as those described above, useful in the present invention may be used to quantitatively or qualitatively detect the presence of wild type or mutant  $\alpha$ 1,2-fucosyltransferase polypeptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the  $\alpha$ 1,2-fucosyltransferase polypeptides are expressed on the cell surface.

The antibodies (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of  $\alpha$ 1,2-fucosyltransferase polypeptides. *In situ* detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody of the present invention. The antibody (or fragment) is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the  $\alpha$ 1,2-fucosyltransferase polypeptide, but also its distribution in the examined tissue. Using the present invention, those skill in the art will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Immunoassays for wild type or mutant  $\alpha$ 1,2-fucosyltransferase polypeptides typically comprise incubating a biological sample, such as a biological fluid, including but not limited to blood, plasma, or blood serum, a tissue extract, freshly harvested cells, or cells which have been incubate in tissue culture, in the presence of a detectably labeled antibody capable of identifying  $\alpha$ 1,2-fucosyltransferase polypeptides, and detecting the bound antibody by any of a number of techniques well known in the art.

Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibody or antibody fragments, it is possible to detect wild type or mutant  $\alpha$ 1,2-fucosyltransferase polypeptides through the use of radioimmunoassays (RIA) (see, for example, Weintraub, Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. It is also possible to label the antibody with a fluorescent compound such fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as  $^{152}\text{Eu}$ . Additionally the antibody may be detected by coupling it to a chemiluminescent compound such as luminol, isoluminol, theramatic acreidinium ester and oxalate ester.

- 5 The following examples are intended to illustrate but not limit the invention. While they are typical, other procedures known to those skilled in the art may alternatively be used to illustrate the embodiments and methods of the invention.

#### **EXAMPLE 1**

10 **Cloning and sequence information of the *H. pylori* fucosyltransferase (*fucT*) gene**

**Bacterial strains and Media.** *H. pylori* strains 26695 and UA802 were used for cloning, sequencing and mutagenesis of *fucT2* genes. *H. pylori* cells were cultured on BHI-YE agar or in BHI-YE broth under microaerobic conditions (Ge and Taylor, 1997, *In Methods in Molecular Medicine*, C.L. Clayton and H. Mobley (eds).

- 15 Totowa, NJ: Humana Press, pp. 145-152). *E. coli* strain DH10B was used for production of recombinant plasmids.

**Cloning of *H. pylori*  $\alpha(1,2)$  fucosyltransferase gene (*Hp fucT2*).** Two primers, GW44 (5'-GAACACTCACACGCGTCTT-3', position 99980-99962 in the published *H. pylori* genome) and GW32 (5'-TAGAATTAGACGCTCGCTAT-3', position 20 98855-98874 in the published *H. pylori* genome) were used to PCR amplify a 1.12 kb fragment containing *Hp fucT2* from *H. pylori* 26695 and UA802 chromosomal DNA. In addition, by using a primer GW43 (5'-CGGAGGGCTTGGGAATCAA-3', position 99814-99796 in the published *H. pylori* genome ) and primer GW32, a PCR fragment of 0.96 kb carrying 5'- truncated *fucT2* gene was obtained from UA802 25 chromosomal DNA. The PCR fragments were directly cloned into pGEM-T vector (Promega) following the manufacturer's instructions. The orientation of the genes cloned in the plasmids was examined by restriction enzyme analysis, and those clones with the *fucT2* gene under the control of T7 promoter were selected. The resultant

plasmids, pGEMB3, pGEMI6, and pGEMH2, are illustrated in Fig. 3A. Subsequently, the genes cloned in the plasmids were sequenced and were shown to be identical to the corresponding genes in the *H. pylori* genome.

- Features of *H. pylori*  $\alpha(1,2)$  *fucT* gene.** Based on the published *H. pylori* genome sequence (Tomb *et al.*, 1997, *Nature* 388:539-547), a pair of primers, GW44 and GW32 (Fig.1A) were designed. These primers were able to PCR amplify a DNA fragment (1.12 kb) from *H. pylori* strain UA802, which corresponds to the region containing HP0094 and HP0093 in 26695. The complete nucleotide sequence of this fragment is 95% identical to that of *H. pylori* 26695. However, it contains a single
- 5 ORF encoding a protein of 300 amino acids with a calculated molecular weight of 35,193 daltons. We designated this gene *Hp fucT2* to distinguish it from the previously identified  $\alpha(1,3)$  *fucT* which was given a name of *fucT* (Martin *et al.*, 1997, *J. Biol. Chem.* 272:21349-21356; Ge *et al.*, 1997, *J. Biol. Chem.* 272:21357-21363). *Hp fucT2* gene has a unique feature in its center region. In addition to a poly
- 10 C tract identified previously (Tomb *et al.* 1997, *Nature* 388:539-547, Berg *et al.* 1997, *Trends Microbiol.* 12:468-474), we identified a sequence of TAA repeats (imperfect, may also be GAA or AAA) immediately following the poly C sequence (Fig. 1B). The changes of the repeat number of the both tracts contribute to the variation of the *fucT2* genotype (on or off status) in different strains (Fig. 1 A, B and Table 2).
- 15 In an attempt to find out the relationship between the *fucT2* gene and the Le<sup>Y</sup> phenotype, six additional *H. pylori* isolates were selected for analysis (Table 2). Together with the strains 26695 and UA802, these total eight strains fall into four groups of Lewis phenotypes: Le<sup>X</sup>-/Le<sup>Y</sup>+, Le<sup>X</sup>+/- Le<sup>Y</sup>+, Le<sup>X</sup>+/-Le<sup>Y</sup>-, and Le<sup>X</sup>-/Le<sup>Y</sup>-. The complete nucleotide sequences of the *fucT2* genes from these strains demonstrated an
- 25 extensive variation in the poly C and TAA repeat sequence among different strains, which make the gene either intact (as in UA802) or frameshifted (as in 26695). Like UA802 *fucT2*, UA1234 *fucT2* encodes an intact ORF, even though there is a deletion of one TAA repeat. The existence of the intact *fucT2* gene in UA802 and UA1234 is



correlated to their Le<sup>Y</sup>+ phenotype. UA1182, another example like 26695, contains a frameshift mutation in its *fucT2* gene. This mutation could be compensated by translation frameshifting, since a *dnaX*-like translation frameshift cassette is present in frame.

5

- The two strains with the Le<sup>X</sup>/Le<sup>Y</sup>- phenotype, UA1174 and UA1218, displayed completely different features in their *fucT2* genes. In UA1174 *fucT2*, there is the insertion of 2C and 2A at the hypermutable region, resulting in a frameshift mutation. Since a *dnaX*-like translation frameshift cassette is absent (the AAAAAAG sequence is not in frame), the frameshift cannot be compensated, giving rise to a Le<sup>Y</sup>- phenotype. On the other hand, UA1218 *fucT2* encodes an intact ORF, because the changes in the hypermutable region do not create a frameshift. However, the result from the PCR and subsequent DNA sequencing revealed a deletion of 80 bp exactly in front of the SD sequence (ribosome binding site) of UA1218 *fucT2* gene.
- 15 Therefore, the Le<sup>Y</sup>- phenotype of UA1218 could be attributed to the absence of the promoter for the expression of the *fucT2* gene. The two strains in the last group, UA1207 and UA1210, have an intact *fucT2* gene, since the deletion of one TAA repeat, or the change of (-C +A), respectively, does not create a frameshift. Therefore, the  $\alpha(1,2)$  FucT in these two strains would be expected to be functional. From the
- 20 Le<sup>X</sup>- phenotype of these strains we can infer that their  $\alpha(1,3)$  FucT may be not functional, which also leads to the Le<sup>Y</sup>- phenotype.

**Table 1. Characterization of *H. pylori fucT2* mutants**

	<i>H. pylori</i> strains <sup>a</sup>	ELISA reactivity (ODU) <sup>b</sup> with MAbs	
		anti-Le <sup>Y</sup>	anti-Le <sup>X</sup>
25	26695	0.477 ± 0.047 (+)	0.414 ± 0.042 (+)
	26695ΔH	0.058 ± 0.014 (-)	0.437 ± 0.016 (+)
	26695ΔE	0.048 ± 0.025 (-)	0.829 ± 0.038 (+)
	UA802	0.63 ± 0.072 (+)	0 ± 0.002 (-)
	UA802ΔH	0.03 ± 0.003 (-)	0.144 ± 0.048 (+)
	UA802ΔE	0.069 ± 0.037 (-)	0.336 ± 0.022 (+)
30	UA802ΔE	0.069 ± 0.037 (-)	0.336 ± 0.022 (+)

- a.  $\Delta H$  or  $\Delta E$  represents *H. pylori* mutants carrying CAT insertion within the *fucT2* gene at *Hind*III or *Eco*RI site.
- b. ELISA reactivity is expressed as the absorbance at 405 nm (ODU) and the values are averaged from triplicate determinations with standard deviation. Using the criteria of Wirth *et al.* (1996), the ODU over 0.1 units are considered positive, and the positivity/negativity is indicated in parentheses.

**Table 2 Correlation of the *fucT2* genotype with the LeY phenotype in various *H. pylori* strains.**

strains	Lewis phenotype <sup>a</sup>		<i>fucT2</i> genotype			proposed gene status <sup>f</sup>		
	LeX	LeY	sequence divergence <sup>b</sup>	ORF <sup>d</sup>	translation frameshift <sup>e</sup>	<i>fucT</i>	<i>fucT2</i>	ratio of FucI/FucT2
UA802	-	+	reference	intact	-	on/off	on	low
UA1234	-	+	-(TAA)	intact	-	on/off	on	low
26695	+	+	+2C	frameshifted	+	on	off/on	high
UA1182	+	+	-C	frameshifted	+	on	off/on	high
UA1174	+	-	+2C, +2A	frameshifted	-	on	off	-
UA1218	+	-	+C, -(AATA), and $\Delta P^c$	intact	-	on	off	-
UA1207	-	-	-(TAA)	intact	-	off	on	-
UA1210	-	-	-C, +A	intact	-	off	on	-

- a: Lewis phenotype is based on the ELISA reading for each strain. For all the strains selected here, “-“ represents the ELISA readings below 0.1 units, and “+” represents those over 0.3.
- b: Listed are the major changes of the sequence in the highly variable region (poly C and TAA repeats) in reference to UA802 *fucT2*. Other base substitutions throughout the whole gene are easily identified to those skilled in the art. The sequences of the *fucT2* genes from these strains have been deposited in the GenBank under the accession number AF076779 (UA802), AF093828 (UA1234), AF093829 (UA1182), AF093830 (UA1174), AF093831 (UA1218), AF093832 (UA1207), and AF093833 (UA1210).
- c:  $\Delta P$  indicates the deletion of a 80 bp-fragment in the promoter region of the gene.

- d: Due to the highly variable sequences, the ORF of *fucT2* gene is either intact (complete) or frameshifted (truncated).
- e: Translation frameshift is predicted based on the sequences of the *dnaX*-like translation frameshift cassette identified in the individual *fucT2* gene.
- 5 f: FucT refers to the  $\alpha(1,3)$ FucT. The model is described in detail in the Discussion.

- Hp fucT2* gene has a unique feature in its center region which is responsible for the occurrence of the variant type of the gene in *H. pylori* 26695. It contains a poly C tract followed by imperfect TAA (or GAA, or AAA) repeats (Fig. 1B). UA802 *fucT2* has a run of 12 Cs, which allows the initiating translation frame (0 frame) be read
- 10 through this region, giving rise to a translation product of full length. In the case of 26695 *fucT2*, the existence of two more Cs (total 14 Cs) leads to early termination of the initiating frame (HP0094) at a TGA stop codon (Fig. 1B). Downstream of HP0094, there appears to be a potential start codon (ATG) in another frame which could be read to generate HP0093 (Fig. 1A).
- 15 Since the poly C tract was identified within the *Hp fucT* genes (both  $\alpha 1,3$ - and  $\alpha 1,2$  *fucT*), it was believed that such simple oligonucleotide repeat regions are hypermutable and could offer an on-off mechanism for the expression of the gene (Saunders *et al.*, 1998, *Mol. Microbiol.* 27:1091-1098), and may therefore be responsible for the phase variation of LPS expression. Indeed, the number of poly C
- 20 repeats in *Hp fucT2* gene is variable among different strains (N=11-14, the reference UA802 *fucT2* has 12 Cs, Table 2). Additionally, we observed that the subsequent TAA repeat sequence (or called A-rich sequence) is also a mutation hotspot. The divergence at these repetitive sequences gave rise to the two types of the gene, encoding either a full-length product (hypothetically gene-on) or a truncated
- 25 product(s) (hypothetically gene-off).

However, certain strains with a hypothetical off-status of the *fucT2* gene have the Le<sup>Y</sup>+ phenotype, as exemplified in 26695. The identification of a nucleotide sequence

resembling the *E. coli dnaX* translation frameshift cassette within the 26695 *fucT2* gene and the result of *in vitro* expression of the gene provide a reasonable mechanism by which the full-length protein could be produced by certain off-status *fucT2* genes. Programmed translation frameshifts appear in genes from a variety of organisms and

5 the frequency of frameshifting can be very high in some genes, approaching 100% (Farabaugh, 1996, *Annu. Rev. Genet.* 30:507-528). The best studied -1 frameshift model is *E. coli dnaX*, the gene for the  $\tau$  subunit of DNA polymerase III. As a result of translation frameshifting, a truncated product ( $\gamma$  subunit of DNA polymerase III) is synthesized from *dnaX* in a frequency of about 40%-50% (Flower and McHenry,

10 1990, *Proc. Natl. Acad. Sci. USA* 87:3713-3717). Both  $\tau$  and  $\gamma$  subunits are required for DNA synthesis, and are needed respectively for leading and lagging strand synthesis, due to their different processivity. The main element in *dnaX* translation frameshift cassette is A AAA AAG heptamer sequence in the appropriate reading frame. It has been shown that the efficient frameshifting at this sequence is due to the

15 absence of tRNA<sup>Lys</sup> with a CUU anticodon in *E. coli* (Tsuchihashi and Brown, 1992, *Genes Dev.* 6:511-519). When the tRNA<sup>Lys</sup> with UUU anticodon encounters the AAG lysine codon it can easily slip to the -1 frame where it interacts with the AAA lysine codon more strongly. From the *H. pylori* whole genome sequence we know that *H. pylori* has only one tRNA<sup>Lys</sup> with a UUU anticodon. In addition, similar to those in

20 the *dnaX* gene, the frameshift-stimulating elements including a putative SD sequence and a stem-loop structure were also found up- and downstream of the A AAA AAG sequence in 26695 *fucT2* gene. Thus, it is very likely that certain *H. pylori* strains like 26695 use the same mechanism as *E. coli dnaX* gene to generate -1 frameshift in translation of their *fucT2* genes. Although we have observed the full-length and half-

25 length protein bands from the *in vitro* expression of 26695 *fucT2* gene, the accurate frequency of translational frameshifting in this gene, as well as in the genes from different stains, has not yet been determined. Also, the expression of the gene in *H. pylori* cells could very well be different from that observed *in vitro* using *E. coli* T7 cell extract.

Unlike  $\alpha$  (1, 3) fucosyltransferases,  $\alpha$  (1, 2) FucTs belong to a more heterogeneous family and display very weak homology. Multiple sequence alignment for eukaryotic and prokaryotic  $\alpha$  (1, 2) FucTs allowed us to identify three highly conserved motifs that may have potential structural and/or catalytic importance. While motif I is  
5 located in the N-terminal stem region, motif II and III are located in the proposed catalytic domain (Fig. 2). By insertion mutagenesis we showed that the disruption of the gene at either the *HindIII* or *EcoRI* site abolishes its function in the synthesis of  $Le^Y$ , suggesting the integrity of the gene is necessary for this function.

Unexpectedly, *H. pylori fucT2* mutants express more  $Le^X$  than the wild type cells.  
10 This finding suggests that  $Le^X$  is the substrate for  $Le^Y$  synthesis in *H. pylori*. In the commonly described mammalian system (Avent, 1997),  $Le^Y$  is produced by addition of fucose through an  $\alpha$  (1, 3) linkage on the H type 2 structure (Fig. 5, the pathway on the left). However, Martin *et al.* (1997) found that H type 2 is not the substrate for *Hp*  $\alpha$  (1, 3) FucT, suggesting that in the synthesis of  $Le^Y$  in *H. pylori*,  $\alpha$  (1, 2)  
15 fucosylation may occur after  $\alpha$  (1, 3) fucosylation (Fig. 5, the pathway on the right). Our results here are in good agreement with this notion. Thus, disruption of  $\alpha$  (1, 2) FucT may result in the accumulation of its substrate,  $Le^X$ . Furthermore, there was a difference in the  $Le^X$  expression between the two mutants carrying mutations at the *HindIII* or *EcoRI* site within *fucT2* gene. The *HindIII* site is in the middle of *fucT2*  
20 (type I) gene; downstream of it there exists a potential small ORF corresponding to HP0093 within which the *EcoRI* site is located. In this ORF, which may be expressed in the *HindIII* mutants, there possibly remains the active site or binding site, which may compete with  $\alpha$  (1, 3) FucT for addition of fucose to make more  $Le^X$ . In contrast, the binding site of  $\alpha$  (1, 2) FucT in the *EcoRI* mutants may be completely disrupted.  
25 Consequently, the increased  $Le^X$  level is more evident.

Since the synthesis of  $Le^Y$  requires both  $\alpha$ (1, 3) FucT and  $\alpha$ (1, 2) FucT activities (Fig. 5), turning off either gene will give rise to a  $Le^Y$ - phenotype, as seen in the strains UA1174, UA1218, UA1207, and UA1210 (Table 2). If both genes are on (or partially

on), the levels of expression of  $Le^X$  and  $Le^Y$  will depend on the ratio of the concentrations (activities) of the two enzymes. Without knowing the actual status of  $\alpha(1, 3)$  *fucT* gene (two copies), we assume that the ratio of  $\alpha(1, 3)/\alpha(1, 2)$  FucT in strains UA802 and UA1234 is low, so that the most (or all) of the  $Le^X$  synthesized by  $\alpha(1, 3)$  FucT was converted to  $Le^Y$  by  $\alpha(1, 2)$  FucT. This is supported by the observation that no  $Le^X$  was detected from wild type UA802, while a low level of  $Le^X$  was detected when the *fucT2* gene was disrupted. In strains 26695 and UA1182, the *fucT2* gene is in an off-on switching status due to the compensation by translational frameshifting. As seen from the *in vitro* expression of 26695 *fucT2* gene and inferred from *E. coli dnaX* gene, the frequency of translation frameshift is presumably around 50%. Thus, the  $\alpha(1, 2)$  FucT activity in 26695 could be lower than that in UA802. Since the *fucT2* knock-out mutant of 26695 produced much more  $Le^X$  than the corresponding UA802 mutant, we assume that the  $\alpha(1, 3)$  FucT activity in 26695 is higher than that in UA802. Taken together, we propose that the ratio of  $\alpha(1, 3)/\alpha(1, 2)$  FucT activity in 26695 is high, which confers ( $Le^X+$ ,  $Le^Y+$ ) phenotype. To confirm this model, the genetic analysis for both copies of the  $\alpha(1, 3)$  FucT gene and comparative determination of the activities of both enzymes from both strains are needed.

**DNA sequencing and databases.** Both strands of appropriate PCR fragments or cloned DNA fragments were sequenced using the Thermo sequenase sequencing kit following the manufacturer's instructions (Amersham Life Science, Inc.). Sequence analyses were performed with the BLAST Program of the National Center of Biotechnology Information (Bethesda, MD). The Wisconsin Package (version 9.0) of the Genetics Computer Group (GCG) (Madison, WI) was used for the editing of sequences.

Compared with the sequences in databases, *Hp fucT2* has homologues in other bacteria: a gene (*wbcH*) within the LPS O-antigen gene cluster of *Yersinia enterocolitica* serotype O:8 (Zhang *et al.*, 1997), and a gene (*epsH*) within the

- plasmid encoded *eps* gene cluster essential for exopolysaccharide biosynthesis in *Lactococcus lactis* (van Kranenburg *et al.*, 1997, *Mol. Microbiol.* 24:387-397). Although both *wbcH* and *epsH* gene products could act as  $\alpha(1,2)$  FucT enzymes as predicted from sequence homology, no experimental evidence for assignment of their
- 5 function is yet available. *Hp fucT2* gene product displays a low level of overall similarity in amino acid sequence to its eukaryotic as well as prokaryotic counterparts, with 18 % identity to human Fut2 (Kelly *et al.*, 1995, *J. Biol. Chem.* 270:4640-4649) and 22 % identity to *Y. enterocolitica* WbcH. However, multiple sequence alignment revealed three blocks of highly conserved amino acid sequences
- 10 within all the  $\alpha(1,2)$  FucTs (motifs I, II, and III in Fig. 2), which may be important for the enzyme function. Among them, motif II is the one proposed to be a part of the nucleotide binding domain by Breton *et al.* (1998, *Glycobiology* 8:87-94). Note that this motif is missing from the deduced amino acid sequence of 26695 *fucT2* gene, because the coding region is between HP0094 and HP0093.
- 15 All eukaryotic  $\alpha(1,2)$  FucTs have a typical domain structure consisting of a short N-terminal cytoplasmic tail, a transmembrane domain, and a stem region followed by a large globular C-terminal catalytic domain (Kleene and Berger, 1993, *Biochim Biophys Acta* 1154:283-325). The three bacterial  $\alpha(1,2)$  FucTs so far identified are shorter than the eukaryotic counterparts, and lack the N-terminal cytoplasmic tail and
- 20 transmembrane domain (Fig. 2A). This is evident from the sequence alignment and by the analysis of the secondary structure of *Hp FucT2* (hydropathy profile) which suggests it is a globular protein without any possible transmembrane domain. The N-terminal cytoplasmic tail and transmembrane domain of eukaryotic FucTs are proposed to have a role in Golgi localization and retention of the enzyme. The
- 25 sequences of bacterial  $\alpha(1,2)$  FucTs suggest that the enzyme is a soluble protein localized in the cytoplasm.

## **EXAMPLE 2**

### **Plasmid constructs and Expression of the *H. pylori fucT* gene**

**Insertion mutagenesis and natural transformation.** Plasmid mutants carrying the disrupted *H. pylori fucT2* gene were created by inserting the chloramphenicol acetyltransferase (CAT) cassette (Wang and Taylor, 1990, *Gene* 94:23-28) at *Hind*III or *Eco*RI site (Fig. 3A). Three mutant plasmids were obtained: pGHC26 (CAT  
5 cassette at *Hind*III site of 26695 *fucT2*), pGEC26 (CAT cassette at *Eco*RI site of 26695 *fucT2*), and pGHC8 (CAT cassette at *Hind*III site of UA802 *fucT2*). Plasmid mutants were introduced into the chromosome of *H. pylori* 26695 and UA802 by a natural transformation procedure. Four *H. pylori fucT2* knock-out mutants were obtained: 26695ΔH, 26695ΔE, 802ΔH, and 802ΔE (Note: There is no *Eco*RI site in  
10 UA802 *fucT2* gene, and 802ΔE was obtained by transforming pGEC26 into UA802.)

**In vitro expression of *Hp fucT2* gene.** The plasmids containing *HpfucT2* genes under the control of the T7 promoter, as well as those plasmids with CAT cassette insertion within the *fucT2* genes, were purified by CsCl gradient ultracentrifugation. The purified supercoiled circular DNA were used as template for *in vitro* expression  
15 of the cloned genes using *E. coli* T7 S30 Extract System (Promega) following the manufacturer's instruction. The expressed gene products were labeled with [<sup>35</sup>S] methionine and analyzed on 0.1% SDS -12% polyacrylamide gel followed by autoradiography.

**Immunoelectron microscopy.** *H. pylori* broth cultures were absorbed onto Formvar-  
20 coated electron microscope grids and washed in phosphate buffer. The samples were incubated with primary anti-Le<sup>Y</sup> MAb isotype IgM (Signet Laboratories, Inc.) and further incubated with goat anti-mouse IgM-10nm colloidal gold conjugate (EY Laboratories, Inc., San Mateo, CA). Positive labeling was determined by the presence of gold particles on unfixed and unstained *H. pylori* cells.

25

**ELISA with *H. pylori* whole cell suspensions.** The primary antibodies used were anti-Le<sup>X</sup> (mAB BG-7) and anti-Le<sup>Y</sup> (mAB BG-8) (Signet Laboratories Inc. Dedham, MA). The secondary antibody was anti-mouse IgG + IgM conjugated to horse-radish



peroxidase (HRP) (Biocan #115 035 068, Mississauga, Ontario) diluted 1:2000. The reaction was stopped with 4 mM sodium azide and the absorbance was recorded at 405 nm using a Titretek Multiscan MC (Helsinki, Finland) microtitre plate reader.

**SDS-PAGE and immunoblot analysis of *H. pylori* LPS.** Proteinase K treated whole  
5 cells extracts of *H. pylori* strains were prepared and subjected to electrophoresis on a stacking gel of 5% acrylamide and a separating gel of 15% acrylamide. LPS on the gel was detected either by silver staining or by immunoblotting. The LPS transferred to nitrocellulose membrane (pore size 0.22 µm, Micron Separations Inc. Westboro  
10 MA) were probed with anti-Lewis structure antibodies (1:100 dilution), and subsequently with goat anti-mouse antibody conjugated to horse radish peroxidase (1:2000 dilution). Blots were developed using an enhanced chemiluminescence kit (Amersham) according to the manufacturer's specifications.

**Both types of *Hp fucT2* gene produce a full-length protein *in vitro*.**

As illustrated in Fig. 3A and described in Experimental Procedures, *Hp fucT2* genes  
15 were amplified by PCR and cloned into the pGEM-T vector under the control of a T7 promoter. The genes cloned into the plasmids are identical to those original genes in the *H. pylori* genome as verified by DNA sequencing. The plasmid pGEMB3 contains 26695 *fucT2* gene; pGEMH2 contains a 5'-truncated 802 *fucT2* gene; and pGEMI6 contains complete 802 *fucT2* gene. Subsequently, the CAT cassette was inserted at the  
20 *HindIII* or *EcoRI* site within the cloned *Hp fucT2* genes to obtain plasmid mutants pGHC26 (CAT cassette at *HindIII* site of 26695 *fucT2*), pGEC26 (CAT cassette at *EcoRI* site of 26695 *fucT2*), and pGHC8 (CAT cassette at *HindIII* site of UA802 *fucT2*). The six plasmid DNAs were used as templates for *in vitro* transcription-translation assays to examine the protein products encoded by the corresponding  
25 genes.

The expressed protein products analyzed on SDS-PAGE are shown in Fig. 3B. The expression of 802 *fucT2* gene (pGEMI6, lane 5) gave rise to a major protein of 33

KD, which is very close to that expected from the deduced aa sequence (35 KD). Three weak bands with smaller molecular weights may result from translations starting at internal ATG codons or from degradation of the full length protein. As expected, 5'-truncated 802 *fucT2* did not produce the full-length protein (pGEMH2, lane 4). For the expression of 26695 *fucT2* (pGEMB3, lane 3), two small proteins of 18 and 13 KD were expected based on the DNA sequence of the gene. However, in addition to a 17 KD protein band which may represent the half-length gene product (HP0094), we observed a full-length (33 KD) protein band. To confirm that this result was not due to a mutation in the cloned gene, the sequence of the actual plasmid DNA (pGEMB3) used for the *in vitro* transcription-translation assay was re-examined, and no change was found compared to the original 26695 *fucT2* gene.

The observation that 26695 *fucT2* gene produces the full-length protein prompted us to consider other possibilities which could account for this result: RNA polymerase slippage in transcription or ribosome slippage in translation. By re-examining the DNA sequences of the simple repeat region within *Hp fucT2* gene (Fig. 1B), we found three motifs (X XXY YYZ) typical of programmed translation frameshift (Farabaugh, 1996) occurred in the appropriate reading frame. The first one (C CCT TTA), located upstream of the poly C tract, exists in 26695 *fucT2*, but not in 802 *fucT2*. The second one (A AAA AAG), located downstream of the poly C tract, is present in the reading frame of 26695 *fucT2*, but not in the reading frame of 802 *fucT2*. This motif is identical to the extremely slippery heptanucleotide found in the mRNA of *E. coli dnaX* (Flower and McHenry, 1990). Other elements of *dnaX* frameshifting signal (Larsen *et al.*, 1994, *J. Bacteriol.* 176:6842-6851) including an upstream SD sequence and a downstream stem-loop structure which serve as stimulators of the frameshifting are also present in the deduced 26695 *fucT2* mRNA sequence (Fig. 1C). Therefore, A similar mechanism for -1 frameshift as in *dnaX* is very likely at work in 26695 *fucT2*: exactly at the site behind the poly-C region where a frameshift has occurred (relative to the prototype 802 *fucT2*) and before encountering the stop codon, the reading frame could be shifted back (at a certain frequency) to the reading frame of the prototype

gene, so that a full-length protein could be produced. Interestingly, just four codons before the HP0093 start codon, there exists another A AAA AAG sequence, both in 26695 and 802 *fucT2* genes (Fig. 1B, line 4). However, no upstream SD sequence and downstream stem-loop structure were found around this slippery sequence.

- 5 Analysis of the *fucT2* genes from several different strains (Table 2) demonstrated the various factors affecting expression of this gene and the ultimate Lewis phenotype. First, some divergence in the promoter region was observed among different strains, which could contribute to the differential expression of the gene through regulating transcription. Although the function of the promoter of *Hp fucT2* gene was not
- 10 performed in detail, apparently in strain UA1218 the promoter was completely missing resulting in the off-status of the gene. Second, two elements within the coding region of the gene were identified that could affect the coding ability of the gene. The first element, the simple sequence repeat region, is a mutation hotspot. As suggested previously (Tomb *et al.* 1997, Berg *et al.* 1997, Saunders *et al.* 1998), the
- 15 frameshift mutation produced by DNA polymerase slippage during the replication of the gene may provide a mechanism for the switching between on and off status of the gene (at a frequency of <1%), which could account for the phase variation of Le<sup>Y</sup> expression reported by Appelmelk *et al.* (1998, *Infect. Immun.* 66:70-76). The extensive sequence divergence at this hypermutable region among various strains and
- 20 the resulting two types (intact or frameshifted) of the gene support the notion that this strand-slippage mechanism occurs in *H. pylori*.

The second element within the gene is the slippery sequence for ribosome translation which is located immediately behind the hypermutable region. In certain strains that

25 have a -1 frameshift mutation (relative to the prototype), such as 26695 and UA1182, the translation could be shifted back to the prototype reading frame at a high frequency, producing functional proteins. In other strains that have a prototype reading frame (*e.g.* UA802) or +1 frameshift (*e.g.* UA1174), this slippery sequence is not in frame, thus is not functional. Therefore, the frameshift mutation in UA1174

*fucT2* cannot be compensated at the translation stage, resulting in the off-status of the gene. Interestingly, in the reading frame of the prototype *fucT2* genes such as that of UA802 there exists another A AAA AAG slippery sequence in frame but without enhancing elements (Fig.1B, line 4). It is not known whether translational  
5 frameshifting occur here at very low frequency to produce a minor fraction of truncated protein. If so, it could affect the level of the Le<sup>Y</sup> production, although insignificantly. In summary, it is propose that translational frameshifting may offer *H. pylori* an mechanism by which the full-length (active) and truncated (inactive or less efficient) enzymes can be produced in various ratios which account for the different  
10 levels of Le<sup>Y</sup> production among various strains. This ratio could also be influenced by certain environmental factors in the course of *H. pylori*-host interaction, leading to the varied level of Le<sup>Y</sup> expression in an individual strain.

**Effect of *fucT2* knock-out mutations on the expression of Le<sup>Y</sup> and Le<sup>X</sup> in *H. pylori*.**

15 To demonstrate the requirement of *Hp fucT2* in the biosynthesis of Le<sup>Y</sup>, we performed insertion mutagenesis of *fucT2*. As described in Experimental Procedures, we constructed four *H. pylori fucT2* knock-out mutants: 26695ΔH, 26695ΔE, 802ΔH, and 802ΔE, in which the *fucT2* gene of *H. pylori* 26695 or UA802 was disrupted by insertion of a CAT cassette at *Hind*III or *Eco*RI site, respectively. The insertion of the  
20 CAT cassette at the specific location of the *fucT2* gene in the *H. pylori* genome was verified by PCR amplification of an expected fragment and by DNA sequencing of the region surrounding the insertion site. The phenotypes of these *H. pylori* mutants for Le<sup>Y</sup> expression were examined by electron microscopy and by ELISA.

Fig. 4 shows an example of the transmission electron micrographs of UA802 wild  
25 type and mutant cells immunostained with anti-Le<sup>Y</sup> MAbs. Wild type cells strongly express Le<sup>Y</sup>, as evidenced by the presence of many gold particles. In contrast, the mutant cells, 802ΔH and 802ΔE (shown here is only 802\_ΔH), were negative for immunogold labeling using anti-Le<sup>Y</sup> antibody. A similar pattern of electron

micrographs for strain 26695 (Le<sup>Y</sup> positive) and its mutants (Le<sup>Y</sup> negative) was observed.

A quantitative examination for the expression of Le<sup>Y</sup> as well as Le<sup>X</sup> by these strains detected by ELISA is given in Table 1. Wild type strain 26695 expresses both Le<sup>Y</sup> and Le<sup>X</sup> (ODU = 0.48 and 0.41, respectively), while wild type UA802 strongly expresses Le<sup>Y</sup> (ODU = 0.63) but no Le<sup>X</sup>. All of their isogenic mutants were negative for Le<sup>Y</sup> (ODU < 0.1), suggesting that disruption of the *fucT2* gene at both *Hind*III and *Eco*RI site abolish Le<sup>Y</sup> expression. Interestingly, there is an increase in the expression of Le<sup>X</sup> for the *fucT2* mutants, especially when the mutation is at the *Eco*RI site.

10 Further characterization of these mutants was carried out by SDS-PAGE and immunoblots of the LPS for detection of Le<sup>Y</sup> and Le<sup>X</sup> (Fig. 5). Silver stained gels revealed no change in the LPS side chain length for all the mutants compared with the wild type cells. The immunoblots confirmed that Le<sup>Y</sup> is expressed by the wild type strains 26695 and UA802, and is no longer expressed in all the *fucT2* mutant strains  
15 (Fig. 5A). Wild type UA802 does not express any Le<sup>X</sup> on its surface, but its isogenic *fucT2* mutants do express Le<sup>X</sup> (Fig. 5B). There was no significant difference on the Le<sup>X</sup> expression levels between the two mutants (802ΔH and 802ΔE), which is different from the ELISA results. Since there is Le<sup>X</sup> expression in the wild type strain 26695, the increase of Le<sup>X</sup> expression in its mutant strains is not so evident. Similar to  
20 the ELISA results, however, a significant increase in Le<sup>X</sup> expression was observed in 26695ΔE, but not in 26695ΔH.

### EXAMPLE 3

#### Enzymatic activities of *H. pylori* α1,2 fucosyltransferase

Overexpression of the *H. pylori* fucosyltransferase in *E. coli*. In a typical  
25 experiment, *E. coli* CLM4 (pGP1-2) cells harboring a plasmid carrying an *H. pylori* *fucT* gene (pBKHp763fucT39, pGEMH2, pGEMI6 or pGEMB3) were grown in 25 ml liquid LB medium with appropriate antibiotics (kanamycin and ampicillin) at

30°C to an optical density of 0.5-0.7 at 600 nm. After being collected, the cells were washed once with M9 medium, resuspended in 5 ml of supplemented M9 medium, and further incubated at 30°C for 1 h. To induce the expression of the *fucT* gene, the cell culture was shifted to 42°C by adding 5 ml prewarmed (55 °C) supplemented M9  
5 medium. After incubation at 42 °C for 15 min, rifampicin was added to a final concentration of 200 µg/ml, and cell growth was continued at 42 °C for 20 min.

For analysis of the protein on SDS-PAGE, a small aliquot (0.5 ml) of the cell culture was taken, and 2.5 µl of [<sup>35</sup>S]-methionine (4.35x10<sup>13</sup> Bq/mmol, 3.7x10<sup>8</sup> Bq/ml, NENT<sup>TM</sup>, Boston, MA) was added. After further growth at 30 °C for 30 min, the cells  
10 were harvested, resuspended in 100µl sample buffer (50 mM Tris-HCl, pH6.8, 1% (w/v) SDS, 20 mM EDTA, 1% (v/v) mercaptoethanol, 10% (v/v) glycerol), and boiled for 3 min before loading on to the gel. For the preparation of the sample for the enzyme assay, the remaining part (major aliquot, 9.5 ml) of the cell culture after induction was further incubated at 30 °C for 30 min, then harvested. The cells were  
15 washed with 1.5 ml of 20 mM HEPES (pH 7.0), and resuspended in 1.5 ml of this buffer supplemented with 0.5 mM PMSF.

Preparation of cell lysates or cell extracts for the fucosyltransferase assay. The *E. coli* cells containing overproduced Hp FucT proteins, which were in HEPES buffer with PMSF as described above, were disrupted with a French press at 7000 lb/in<sup>2</sup> at 4 °C.  
20 The cell lysates were used directly for enzyme assays. For determining the location of the enzyme activities, the cytoplasmic and membrane fractions were separated as follows. The cell lysates were centrifuged at 13,000xg at 4 °C for 10 min. The cell debris were discarded and the supernatant was subjected to ultracentrifugation at 128,000 xg (Beckman TL100/rotor 100.2) at 4 °C for 1h. The supernatant was  
25 collected as the cytoplasmic fraction. The membrane pellets were resuspended in a small volume of the same buffer and treated with 1 M NaCl.

**Fucosyltransferase assay.** Assays of Hp  $\alpha$ 1,2 and  $\alpha$ 1,3 FucT activities were carried out according to the method described by Chan *et al.* (1995, *Glycobiology* 5:683-688) with some modifications. Reactions were conducted at 37 °C for 20 min in a volume of 20  $\mu$ l containing 1.8 mM acceptor, 50  $\mu$ M GDP-fucose, 60000 dpm

5 GDP-[ $^3$ H]fucose, 20 mM HEPES buffer (pH7.0), 20 mM  $MnCl_2$ , 0.1 M NaCl, 35 mM  $MgCl_2$ , 1 mM ATP, 5 mg/ml BSA, and 6.2  $\mu$ l of the enzyme preparation. The acceptors used in this study were: LacNAc [ $\beta$ Gal 1-4  $\beta$ GlcNAc],  $Le^x$  [ $\beta$ Gal 1-4 ( $\alpha$  Fuc1-3)  $\beta$ GlcNAc], Type 1 [ $\beta$ Gal 1-3  $\beta$ GlcNAc], and  $Le^a$  [ $\beta$ Gal 1-3 ( $\alpha$  Fuc1-4)  $\beta$ GlcNAc]. GDP-[ $^3$ H]fucose ( $1.9 \times 10^{11}$  Bq/ml /mmol) was obtained from American

10 Radiolabeled Chemicals Inc. (St. Louis, MO). Sep-Pak Plus C-18 reverse-phase cartridges were purchased from Waters (Mississauga, ON). For calculation of the specific activity of the enzyme (micro-units per milligram protein), protein concentrations of the cell extracts were determined with a BCA protein assay kit (Pierce, Rockford, IL) using BSA as a standard according to the supplier's

15 instructions.

**Acceptor specificity of Hp  $\alpha$ 1,2 FucT.** Plasmid pGEMI6 carries the prototype fucT2 gene from *H. pylori* UA802 which produces a high level of  $Le^y$ . Initially, we quantitated the  $\alpha$ 1,2 FucT activity by using LacNAc and  $Le^x$  as acceptors, the two potential substrates of  $\alpha$ 1,2 FucT for the synthesis of  $Le^y$  (Fig.5). Surprisingly, almost

20 no activity was detected using LacNAc as an acceptor, whereas considerable activity was observed for the monofucosylated  $Le^x$  acceptor (Table 3B). The specific activity of  $\alpha$ 1,2 FucT is much lower compared to that of  $\alpha$ 1,3 FucT (Table 3A).

In mammalian cells, the same  $\alpha$ 1,2 FucT enzyme (H or Se, tissue-specific) is normally responsible for the synthesis of both H type 1 and H type 2 structures

25 (Sarnesto *et al.*, 1990, *J. Biol. Chem.* 265:15067-15075; Sarnesto *et al.*, 1992, *J. Biol. Chem.* 267:2732-2744). To determine whether the Hp $\alpha$ 1,2 FucT is also involved in the synthesis of  $Le^b$ , we measured its activity with type 1 oligosaccharide acceptors (Table 3B). Even though UA802 does not express type 1 Lewis antigen, its  $\alpha$ (1,2)

FucT enzyme can transfer fucose to Type 1 and Le<sup>a</sup> acceptors. Compared to Le<sup>x</sup>, type 1 and Le<sup>a</sup> are even more efficient substrates for Hp  $\alpha$ 1,2 FucT (2-fold more active). Thus, Hp  $\alpha$ 1,2 FucT can also synthesize H type 1 and Le<sup>b</sup>.

**Table 3. Activity and acceptor specificity of *H. pylori* fucosyltransferases**

	Overexpressed protein <sup>a</sup> (plasmid construct)	acceptor	proposed product	specific activity ( $\mu$ U/mg) <sup>b</sup>	relative activity (%) <sup>c</sup>
5 A	$\alpha$ 1,3 FucT (pBKHp763fucT39)	LacNAc	Le <sup>x</sup>	1480	
B	$\alpha$ 1,2FucT (UA802) (pGEMI6)	LacNAc	H type 2	14 $\pm$ 8	4.5
		Le <sup>x</sup>	Le <sup>y</sup>	150 $\pm$ 33	49
		Type 1	H type 1	309 $\pm$ 28	100
		Le <sup>a</sup>	Le <sup>b</sup>	301 $\pm$ 13	97

a: *E. coli* whole cell extract containing the overexpressed *H. pylori* FucT protein was used for the enzyme assay.

b: A micro-unit (mU) of the enzyme is expressed as the amount of enzyme that  
10 converts 1 pmol of acceptor to product per min. Specific activity was obtained by dividing the total activity (mU) by the total protein content (mg) in the whole cell extract. The data were averaged from at least three independent determinations with standard deviation as shown.

c: % activity relative to that of UA802  $\alpha$ 1,2 FucT on its best substrate Type 1.

**15 Analysis of the reaction products of Hp  $\alpha$ 1,2 FucT by capillary electrophoresis.**

The reaction products synthesized from different acceptors by the Hp  $\alpha$ 1,2 FucT were further characterized by capillary electrophoresis with laser-induced fluorescence detection. The reaction mixture contained the overproduced UA802  $\alpha$ 1,2 FucT protein (from pGEMI6 clone), GDP-fucose, and different acceptors labeled with

20 tetramethylrhodamine (TMR). The results (Fig. 7) confirmed the data from the



enzyme assay using radioactive labeled GDP-fucose (Table 3B) by identifying the products of the reactions.

When using LacNAc as an acceptor (Fig.7A, line a), no reaction product representing H type 2 was observed, suggesting that LacNAc is not a substrate for Hp  $\alpha$ 1,2 FucT.

- 5 In the reaction using Le<sup>x</sup> as an acceptor (Fig.7A, line b), a small new peak was produced, which co-migrated with a synthetic Le<sup>y</sup>-TMR (standard Le<sup>y</sup>) in the electropherogram, indicating that this new peak represents the Le<sup>y</sup> product synthesized from Le<sup>x</sup> by Hp  $\alpha$ 1,2 FucT. Similarly, by using Type 1 or Le<sup>a</sup> as acceptors (Fig.7B), new peaks co-migrating with authentic products, H type 1 or Le<sup>b</sup> 10 respectively, were observed. As negative controls, the protein extract from the *E. coli* CLM4 (pGP1-2) clone containing the pGEM vector without *Hp fucT2* gene was used in the reactions for each acceptor tested above, and no peaks for the products of  $\alpha$ 1,2 FucT were observed.

- Novel  $\alpha$ 1,2 fucosyltransferase.** Determination of activities of the responsible 15 fucosyltransferases is direct proof to distinguishing between the two possible pathways (Fig.5). The observation in this study that Le<sup>x</sup> but not LacNAc is the substrate for the Hp  $\alpha$ 1,2 FucT clearly indicated that *H. pylori* prefers to use the Le<sup>x</sup> pathway to synthesize Le<sup>y</sup> (Fig. 8A). Other supporting evidence came from the enzyme assay for Hp  $\alpha$ 1,3 FucT: (i) LacNAc is an excellent substrate for Hp  $\alpha$ 1,3 20 FucT (Ge *et al.*, 1997; Martin *et al.*, 1997; and Table 3A); and (ii) Martin *et al.* (1997) found that H type 2 was not the substrate of an Hp  $\alpha$ 1,3 FucT. It should be noted, however, that the fucosyltransferases from different *H. pylori* strains may have different acceptor specificity. Further studies on combined analysis of the  $\alpha$ 1,3 and  $\alpha$ 1,2 FucTs from various *H. pylori* strains are needed to elucidate whether this novel 25 pathway for the synthesis of Le<sup>y</sup> is general in *H. pylori* or is strain-specific.

In addition to its function in Le<sup>y</sup> synthesis, Hp  $\alpha$ 1,2 FucT is also active on type 1 Lewis structures (summarized in Fig. 8B). This provides a basis for the recent finding

that Type 1 ( $\text{Le}^c$ ), H type 1, and  $\text{Le}^a$  are expressed in certain *H. pylori* strains ( $\text{Le}^b$  was also detected in some strains by serological methods but has not yet been confirmed by structural analysis) (Monteiro *et al.*, 1998, *J. Biol. Chem.* 273:11533-11543). Here again, the activity of the Hp  $\alpha 1,2$  FucT to synthesize  $\text{Le}^b$  from  $\text{Le}^a$  indicated that this  
5 bacterial enzyme is different from the normal mammalian counterparts which cannot use  $\text{Le}^a$  as substrate. To know if  $\text{Le}^b$  can be synthesized from H type 1 in *H. pylori* awaits the detection of an  $\alpha 1,4$  FucT. The  $\alpha 1,2$  FucT characterized in this study is from *H. pylori* strain UA802 which does not produce any type 1 Lewis antigen. This suggests that the same  $\alpha 1,2$  FucT enzyme could be used in the strains that produce  
10 type 1 epitopes. The failure to produce type 1 Lewis antigens in many *H. pylori* strains could be due to the inavailability of one of the other enzymes involved in the synthesis of Lewis antigens such as galactosyltransferase that adds  $\beta\text{Gal}$  to  $\text{GlcNAc}$  or  $\alpha 1,3/4$  FucT that places the  $\alpha\text{Fuc}$  unit at  $\beta\text{GlcNAc}$ .

In summary, in contrast to the normal mammalian  $\alpha 1,2$  FucT (H or Se enzyme), Hp  
15  $\alpha 1,2$  FucT prefers to use Lewis X [ $\beta\text{Gal}$  1-4 ( $\alpha\text{Fuc}$ 1-3)  $\beta\text{GlcNAc}$ ] rather than  $\text{LacNAc}$  [ $\beta\text{Gal}$  1-4  $\beta\text{GlcNAc}$ ] as a substrate, suggesting that *H. pylori* uses a novel pathway (via Lewis X) to synthesize Lewis Y. Hp  $\alpha 1,2$  FucT also acts on type 1 acceptor [ $\beta\text{Gal}$  1-3  $\beta\text{GlcNAc}$ ] and Lewis a [ $\beta\text{Gal}$  1-3 ( $\alpha\text{Fuc}$ 1-4)  $\beta\text{GlcNAc}$ ], which provides *H. pylori* with the potential to synthesize H type 1 and Lewis b epitopes.  
20 The ability to transfer fucose to a monofucosylated substrate (Lewis X or Lewis a) makes Hp  $\alpha 1,2$  FucT distinct from normal mammalian  $\alpha 1,2$  FucT.

**Hp  $\alpha 1,2$  FucT is a soluble protein.** DNA sequence analysis predicted the Hp  $\alpha 1,2$  FucT to be a hydrophilic protein, and the same is true for Hp  $\alpha 1,3$  FucT (Ge *et al.*, 1997). However, the determination of Hp  $\alpha 1,3$  FucT activity from the overexpressed  
25 proteins demonstrated that the majority of the activity were present in the membrane fraction (Ge *et al.*, 1997). To delineate the cellular location of the Hp  $\alpha 1,2$  FucT activity, cytoplasmic and membrane fractions of *E. coli* cells overproducing Hp  $\alpha 1,2$  FucT proteins were prepared as described in Materials and Methods. The activity in

both fractions was determined using Le<sup>x</sup> or Type 1 as acceptors (Table 4). There was no detectable activity in the membrane fraction when using Le<sup>x</sup> as an acceptor. By using Type 1 as an acceptor, a very low amount of activity (negligible) was detected in the membrane fraction, which accounts for less than 3% of the total activity. These results indicated that Hp  $\alpha$ 1,2 FucT is a soluble cytoplasmic protein.

**Table 4. Enzyme activities of *H. pylori*  $\alpha$ 1,2 FucT in cytoplasmic and membrane fractions.**

Exp. No.	Acceptor used	protein fraction <sup>a</sup>	specific activity ( $\mu$ U/mg protein)	total activity ( $\mu$ U) <sup>b</sup>	relative activity (%) <sup>c</sup>
1	Le <sup>x</sup>	cytoplasm	38	49	100
		membrane	0	0	0
2	Le <sup>x</sup>	cytoplasm	41	54	100
		membrane	0	0	0
	Type 1	cytoplasm	78	108	100
		membrane	8	3	3

a: As a typical experiment, each protein sample was prepared from 25 ml cell culture of *E. coli* CLM4 (pGP1-2, pGEMI6) containing the overexpressed UA802  $\alpha$ 1,2 FucT. The cytoplasmic and membrane fractions were separated as described.

b: Total activity (micro-units) in each protein sample derived from 25 ml cell

15 culture.

c: Percentage of the total activity in the cytoplasmic or membrane fraction.